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McBindall—A Better Name for CCAAT/Enhancer Binding Proteins?

Minireview

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C/EBP-related transcription factors regulate the balance between cell proliferation and mitotic growth arrest during terminal differentiation. Three new studies give evidence that this regulation is mediated by protein:protein interactions completely distinct from the role of C/EBPs in gene expression.

In the mid-1980s Barbara Graves and Peter Johnson were postdoctoral fellows in my laboratory at the Carnegie Embryology Department. Barbara and Peter were working to purify what they believed to be two independent transcription factors isolated from rat liver tissue. Barbara was chasing an activity designated CCAAT binding protein (CBP) by use of a DNaseI footprinting assay that monitored binding of the protein to a *cis*-regulatory sequence common to many promoters transcribed by RNA polymerase II. Using similar methods, Peter was on the trail of an activity called enhancer binding protein (EBP) that bound with high affinity to the enhancer core element common to the transcriptional enhancers of SV40, murine sarcoma virus, and polyomavirus. Much to the chagrin of Barbara and Peter, as the two efforts matured, the CBP and EBP activities copurified—all the way down to a single polypeptide (Graves et al., 1986; Johnson et al., 1987). My attitude of inveterate optimism gave the following spin—if this single DNA binding protein were capable of binding both of the most celebrated *cis*-regulatory elements associated with RNA polymerase II promoters and enhancers, the activity simply had to be of huge biological relevance. Barbara and Peter were more sanguine and level headed, realizing that this enigmatic DNA binding activity was breaking one of the cardinal rules of gene-specific transcription factors. Instead of binding DNA with high specificity, it was equally adept in the recognition of apparently unrelated substrates. It was in that setting that, behind my back, Barbara and Peter began referring to this activity as either McBindall or McScription factor.

With a bit of arm-twisting, Graves and Johnson were OK with a new name for the factor—C/EBP (CCAAT/enhancer binding protein). More importantly, with the help of Bill Landschulz, they were convinced to continue the task of purification and identification of the relevant polypeptide, culminating with the cloning of a cDNA copy of its encoding gene (Landschulz et al., 1988). In retrospect I trust that Graves, Johnson, and Landschulz are proud of their efforts. Studies of C/EBP led to the discovery of the leucine zipper (bZIP) and basic helix-loop-helix (bHLH) categories of transcription factors, the recognition that the Fos and Jun proto-oncogenes encode a heterodimeric transcription factor, as well as recognition that the Myc proto-oncogene encodes a transcription factor. Such studies also provided a framework and powerful battery of reagents useful for the biological study of C/EBP itself. Finally, biochemical studies of C/EBP helped dispel the myth, anticipated from earlier work on bacterial gene expression, that eukaryotic transcription factors must have exquisite binding specificity. This shift of thinking led to the understanding that eukaryotic genes employ complex enhancers that integrate the instruction of multiple transcription factors acting in a combinatorial manner (Thompson and McKnight, 1992).

The previous issue of *Cell* and the most recent issue of *Molecular Cell* present three exciting papers relevant to the function of various members of the C/EBP family of transcription factors. What readers will see from these new discoveries is that the term McBindall may indeed have been far superior to the silly acronym that I continue to wear around my neck like a rotting albatross. Not only do these transcription factors bind to a variety of different *cis*-regulatory DNA sequences, but they also bind and control the activity other key regulatory proteins—some having nothing to do with gene expression!

Before turning to this new and exciting work a bit more history is useful. In thinking about the biological role of the founding member of the C/EBP family—now designated C/EBPα—we were most influenced by its pattern of cell-type distribution as assessed by the use of high-affinity antibodies. Immunohistochemical staining revealed C/EBPα expression limited to the fully differentiated cells of the tissues in which it was expressed (liver, gut, adipose, skin, mammary gland, etc.). Moreover, in the 3T3-L1 model of terminal adipogenesis, C/EBPα was observed to be absent in undifferentiated preadipocytes, yet copiously expressed in fully differentiated, fat-laden adipocytes (Birkenmeier et al., 1989). These clues led to the idea that C/EBPα might be involved in specification of either mitotic growth arrest, terminal differentiation, or both. In the ensuing 15 years, extensive and articulate studies of C/EBPα in a variety of experimental settings have provided compelling evidence favoring its role in both growth arrest and terminal differentiation. In the former case, Umek and colleagues prepared a stable cell line expressing a chimeric C/EBPα-estrogen receptor fusion protein. Exposure of these cells to estradiol led to cessation of mitotic growth (Umek et al., 1991). In the latter case, Lane and colleagues conducted both loss-of-function and gain-of-function experiments consistent with the essential role of C/EBPα in the terminal differentiation of cultured 3T3-L1 preadipocytes (Lin and Lane 1992, 1994). Both of these fundamental interpretations derived from cell culture experimentation have been rigorously confirmed by the C/EBPα gene knockout studies of Darlington and colleagues (Wang et al., 1995).

Finally, it is notable that mammals encode two additional transcription factors highly related to C/EBPα—designated C/EBPβ and C/EBPδ. The bZIP DNA binding domains of the three proteins are exceedingly similar in

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primary amino acid sequence. Despite this similarity, evidence has accumulated indicating that the latter isoforms may be functionally distinct from C/EBPα when viewed from a biological perspective. Although all three proteins bind DNA as homodimeric transcription factors in a nearly indistinguishable manner, they are expressed with quite distinct temporal kinetics as a function of terminal cell differentiation. In the 3T3-L1 model of terminal adipocyte differentiation, C/EBPβ and C/EBPδ are expressed during the early, clonal expansion phase of adipogenesis. Subsequent to this period of aggressive mitotic cell growth, C/EBPβ and C/EBPδ expression levels diminish acutely and come to be replaced by C/EBPα (Cao et al., 1991). Yeh and colleagues have provided evidence that this temporal cascade of C/EBP isoform expression lies at the heart of both the proliferative and maturational phases of adipogenesis (Yeh et al., 1995).

How is it that certain isoforms of the C/EBP family favor mitotic growth, whereas another exerts an antagonistic influence? Here we are able to turn to three new papers that delve into the precise mechanisms by which C/EBP isoforms differentially regulate mitotic cell growth. Two of these papers provide evidence relevant to the means by which C/EBPα causes mitotic growth arrest. The third paper shows how phosphorylation of C/EBPβ leads to cell survival under conditions of liver regeneration.

Thanks to the magical work of Hartwell, Nurse, Matsui, Mahler, Hunt, Ruderman, and scores of others—recognized just this week by our paragons in Stockholm—we understand the regulatory apparatus responsible for controlling the cell division cycle. In brief, a protein kinase designated cdk2 must be activated in order for cells to move their way through mitosis. Activation of cdk2 falls under the regulation of the famous cyclin polypeptides, whose levels ebb and flow under careful regulation at the levels of both synthesis and degradation. Given the antimitotic activity of C/EBPα, any discerning scientists would happily consider the idea that this transcription factor might regulate cell cycle progression by controlling the transcription of one or more of the genes encoding the master regulatory polypeptides (cyclins, cdks, etc.). No, this is not at all how things work. Wang and colleagues instead show that a short region of the C/EBPα polypeptide interacts directly with the cdk2 and cdk4 enzymes in a manner that prevents cyclin binding (Wang et al., 2001 [October issue of *Molecular Cell*]). This inhibitory interaction is completely independent of the DNA binding domain of C/EBPα and maps to a region of the polypeptide totally distinct from the region that—as will be articulated subsequently—leads to E2F repression. On its own, this short region of C/EBPα can inhibit cdk's in living cells, resulting in growth arrest. Finally, hepatocyte extracts derived from C/EBPα knockout mice contain elevated cdk levels. Going back to the original Umek paper describing C/EBPα-mediated growth arrest, it was shown that the amino-terminal region—now known to inhibit cyclin interaction with cdks—is essential for growth arrest (Umek et al., 1991). At that time the most logical interpretation was that the amino-terminal region represented an essential domain of the transcription factor in the context of its role in gene regulation. Here we offer students an important lesson in biology—parsimony, logic, and theory in biology always take a back seat to experimentation. One would never have guessed that a transcription factor would mediate growth arrest via circuitry having nothing to do with gene expression!

Not being enough that C/EBPα evolved a means of inhibiting cyclin:cdk interaction, Porse and colleagues report the critical nature of C/EBPα as a repressor of E2F in the terminal differentiation of adipocytes and granulocytes (Porse et al., 2001 [October 19 issue of *Cell*]). Recently published studies from Slomiany and Johansen gave evidence of direct repression of E2F-dependent transcription by C/EBPα (Slomiany et al., 2000; Johansen et al., 2001). Porse and colleagues report the generation of laboratory mice bearing lesions in the C/EBPα polypeptide that eliminate E2F repression, yet leave intact C/EBPα's ability to bind DNA and activate its conventional array of target genes. Consistent with in vitro studies showing that these E2F repression-deficient forms of C/EBPα are incapable of suppressing mitotic proliferation, mice bearing similarly impaired forms of C/EBPα were found to be deficient in the terminal differentiation of adipocytes and granulocytes. Such studies provide compelling evidence that E2F repression by C/EBPα is critical for its ability to induce terminal differentiation, and further establish a concrete interpretation of the means by which a lineage-instructive transcription factor can couple growth arrest to differentiation.

Is C/EBPα a tumor suppressor gene? The answer to this question came as a resounding yes from the recent work of Tenen and colleagues (Pabst et al., 2001). By use of standard molecular biological approaches, Tenen discovered dominant-negative mutations in the human gene encoding C/EBPα in acute myeloid leukemias. p53 this is not. On the other hand, these incipient, human genetic studies do provide evidence favoring the validity of scientific interpretations of C/EBPα function as studied in model systems.

Saving the best for last, we now turn to the work of Buck and colleagues on C/EBPβ (Buck et al., 2001 [October issue of *Molecular Cell*]). Recall that C/EBPβ, unlike C/EBPα, is expressed in proliferative cells—at least as studied in cell culture systems of terminal differentiation (Cao et al., 1991; Yeh et al., 1995). The Buck study started with a model for hepatic injury. Under such conditions, including exposure to carbon tetrachloride, hepatic stellate cells produce excessive fibrous tissue leading to cirrhosis. It was observed that carbon tetrachloride induces activation of the RSK protein kinase, which in turn leads to stellate cell proliferation and phosphorylation of threonine 217 of C/EBPβ. By contrast, the same treatment of C/EBPβ-deficient mice caused stellate cells to suffer programmed cell death. Remarkably, mice bearing a threonine-to-alanine mutation at residue 217 of the C/EBPβ polypeptide respond to liver injury in the same manner as C/EBPβ-deficient animals.

What is so important about phosphorylation of threonine 217 of C/EBPβ? Buck and colleagues argue that this modification creates a functional XEXD caspase substrate inhibitor. KphosphoTVD is interpreted to form an active caspase inhibitor/substrate—which could be mimicked by a gain-of-function allele of C/EBPβ wherein threonine 217 was changed to glutamic acid. Finally, Buck and colleagues provide evidence that hepatic stel-

late cells derived from C/EBPβ-deficient and C/EBPβ-ala217 mice can be rescued from programmed cell death by treatment with the cell permeant $KE^{217}VD$ peptide or a C/EBPβ-glu217 expression vector. What does this all mean? Taken most literally, the data of Buck and colleagues say that RSK-mediated phosphorylation of threonine 217 of the C/EBPβ transcription factor constitutes a critical event allowing stellate cells to evade programmed cell death upon liver injury. This has nothing whatsoever to do with the role of C/EBPβ as a transcription factor—but represents a literal life-or-death reaction for stellate cells.

Are these "out of the box" activities of C/EBP polypeptides baroque exceptions to our ordering thinking that gene products have evolved to efficiently accommodate highly individualized tasks? By contrast, are we instead seeing the tip of an unanticipated iceberg? Might it be that biological reactions and pathways are far more interwoven than one would logically anticipate? If so, chalk one more point up for the experimentalists. The smartest theoretician—even if qualified as a card-carrying "nonlinear thinker"—could never have predicted the outcomes we now so pleasurably contemplate.

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